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Insulin-Like Growth Factor-I Concentration in the Follicular Fluid of Bali Cattle and Its Role in the Oocyte Nuclear Maturation and Fertilization Rate

H. Hasbi[a,b], S. Gustina[b,c], N. W. K. Karja[b], I. Supriatna[b], & M. A. Setiadi[b,*]
[a]Department of Animal Production, Faculty of Animal Science, Hasanuddin University
Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
[b] Division of Reproduction and Obstetric, Department of Veterinary Clinic, Reproduction and Pathology,
Faculty of Veterinary Medicine, Bogor Agricultural University
Jalan Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia
[c]Division of Animal Science, Faculty of Animal Science and Fisheries, Sulawesi Barat University
Majene, Sulawesi Barat, Indonesia
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ABSTRACT

The objective of this study was to determine the concentration of IGF-I in the follicular fluid (FF) of Bali cattle and its role in the nuclear maturation and fertilization rate. The follicular fluid was collected by the aspiration technique, then it was centrifuged at 1500 g for 30 min at 24°C. The supernatant was collected and stored at -20°C until being used in the experiment for analysis of IGF-1. A total of 1105 oocytes were used in this study. The oocytes were matured in M199 without supplementation of bovine serum albumin, with supplementation of BSA, and with supplementations of 10% FF (v/v) from the follicle with diameter Ø<4 mm, 4≤Ø<6 mm, 6≤Ø<8 mm, and Ø≥8 mm at the luteal phase and then fertilized. The results showed that the concentrations of IGF-I in the FF obtained during the luteal phase was significantly higher (P<0.05) compared to those obtained during follicular phase. The IGF-I concentrations in the follicular fluid of follicle with diameter smaller than 6 mm were significantly higher (P<0.05) compared to those with diameters larger than 6 mm. The percentage of nuclear maturation rate of oocytes cultured with FF obtained from follicle with diameter <4 mm was significantly higher (P<0.05) compared to those obtained from the other groups of follicle diameters. The supplementation of maturation media with BSA and FF were able to improve fertilization rate significantly (P<0.05) compared to maturation media without BSA. In conclusion, the concentration of IGF-I in the follicular fluid obtained during the luteal phase was higher compared to those obtained during the follicular phase. The IGF-I concentrations in the follicular fluid of smaller follicles (diameter <6 mm) were higher compared to those in the large follicles (diameter ≥6 mm). The supplementation of FF can improve the nuclear maturation and fertilization rate.

Keywords: IGF-I, luteal, follicular, Bali cattle, oocytes

ABSTRAK

Penelitian ini bertujuan untuk menentukan konsentrasi IGF-I cairan folikel sapi Bali dan pengaruhnya pada angka maturasi inti dan fertilisasi. Cairan folikel dikumpulkan dengan teknik aspirasi, selanjutnya disentrifugasi pada kecepatan 1500 g selama 30 menit pada suhu 24°C. Supernatan dikoleksi selanjutnya disimpan pada suhu -20°C hingga digunakan untuk penelitian dan untuk analisis IGF-I. Total oosit yang digunakan pada penelitian ini adalah 1105. Oosit dimaturasi dalam M 199 tanpa penambahan BSA, dengan penambahan BSA, dengan penambahan 10% cairan folikel yang berasal dari fase luteal dengan diameter folikel Ø<4 mm, 4≤Ø<6 mm, 6≤Ø<8 mm, dan Ø≥8 mm yang selanjutnya difertilisasi. Hasil penelitian menunjukkan bahwa konsentrasi IGF-I cairan folikel yang berasal dari fase luteal nyata lebih tinggi (P<0.05) dibandingkan dengan cairan folikel yang berasal dari fase folikuler. Konsentrasi IGF-I dalam cairan folikel berdiameter <6 mm nyata lebih tinggi (P<0.05) dibandingkan dengan cairan folikel berdiameter >6 mm. Tingkat maturasi inti oosit yang dimaturasi dengan penambahan cairan folikel berdiameter <4 mm nyata lebih tinggi (P<0.05) dibandingkan dengan kelompok yang lain. Penambahan BSA dan cairan folikel secara nyata meningkatkan angka fertilisasi (P<0.05) dibandingkan dengan tanpa penambahan BSA. Kesimpulan, konsentrasi IGF-I dalam cairan folikel yang berasal dari fase luteal lebih tinggi dibandingkan dengan yang ber-

*Corresponding author:
E-mail: setiadi03@yahoo.com

asal dari fase folikuler. Konsentrasi IGF-I dalam cairan folikel berdimater kecil (diameter <6 mm) lebih tinggi dibandingkan dengan pada folikel berdiameter besar (diameter ≥8 mm). Penambahan cairan folikel dapat meningkatkan angka maturasi inti dan fertilisasi.

Kata kunci: IGF-I, luteal, folikuler, sapi bali, oosit

INTRODUCTION

Follicular fluid (FF) is a natural media for the nuclear and cytoplasmic maturation and ovulation of mammalian oocytes *in vivo* (Coleman *et al*., 2007; Revelli *et al*., 2009), which is the result of a complex fluid secretion by the follicle (Nandi *et al*., 2008). Coleman *et al*. (2007) described that during the growth of oocyte, metabolic processes were occurred in the oocyte and follicular cells. Follicular cells (granulosa and theca cells) produce a number of steroids and certain types of proteins, including growth factors. FF consists of a variety nutrients, growth factors, hormones, electrolytes, metabolites, enzymes, and regulatory molecules that play critical roles in the development and maturation of oocytes (Nandi *et al*., 2008; Sinclair *et al*., 2008; Bender *et al*., 2010; Petro *et al*., 2012). FF protects oocyte from factors leading to premature resumption of meiosis, prevents proteolytic process, facilitates the extrusion of oocyte during ovulation, and enhances sperm attraction, motility, and acrosome reaction (Avery *et al*., 2003).

Insulin-like growth factor-I (IGF-I) is one of complex components of IGF superfamily which plays an important role in mammalian reproduction and is produced in the hypothalamus, ovaries, oviducts, and uterus (Velazquez *et al*., 2008). The IGF-I in the follicular fluid is mainly produced by the follicle and corpus luteum (Woad *et al*., 2000; Velazquez *et al*., 2008), and its concentration increases progressively during follicular growth until the dominant follicle is ovulated. It was reported that the average concentration of IGF-I in follicular fluid obtained from the small follicle was lower than that obtained from the large follicle of porcine (Oberlender *et al*., 2013). Furthermore, Silva *et al*. (2009) explain that the IGF-I plays an important role in the development of primordial follicles to pre-ovulatory follicles. Insulin-like growth factor-I acts to initiate the growth of primordial follicles to primary follicles and the growth of primary follicles to secondary follicles. At the stage of follicle development from secondary follicle to antral follicle, IGF-I controls the follicle growth and survival, proliferation and differentiation of granulosa cells, the production of estradiol and induces the expression of FSH-R on the granulosa cells, the cells survival, and the formation of cortical granules. During the stage of development from the antral to pre-ovulatory follicles, IGF-I controls the proliferation of the granulosa cells, the production of estradiol and progesterone by the granulosa cells, increases the sensitivity of the follicle to gonadotropins, viability of oocytes, increases the LH-R on granulosa and theca cells, the dominant follicle and multiple ovulation, oocyte maturation, and secretion of inhibin A, activin A, and folistatin by the granulosa cells.

The use of follicular fluid for *in vitro* embryos production is rather contradictive. Follicular fluid as a maturation media had an inhibitory effect on germinal vesicle breakdown, meiotic progression, and nuclear maturation (Ducolomb *et al*., 2013). In contrary, numerous studies reports that the supplementation of maturation media with follicular fluid have beneficial effects on the oocyte development competence (Ito *et al*., 2008; Agung *et al*., 2010), promotes sperm penetration during *in vitro* fertilization (Somfai *et al*., 2012) and embryo quality (Valckx *et al*., 2015). However, the use of commercial IGF-I in maturation medium requires the high cost for *in vitro* embryo production.

In vivo, IGF-I in follicular fluid induces mitotic division in granulosa cells (Spanos *et al*., 2000) and resume meiosis to stimulate the formation of LH receptors on the granulosa cells that cause the follicle more responsive to LH (Hurk & Zhao, 2005). Therefore, the aim of the present study was to determine the concentration of IGF-I in follicular fluid obtained from different diameters of follicles and its role in oocyte nuclear maturation and fertilization rate. In this experiment we used Bali cattle as a source of Indonesian original germplasm. Bali cattle is characterized with the high reproductive performance, good carcass quality, and adaptive to the tropical environmental conditions (Supriyantono *et al*., 2008).

MATERIALS AND METHODS

Collection, Preparation, and Analysis of IGF-I Concentration in FF

The ovaries of Bali cattle were collected from local abattoir. The collected ovaries were transported to the laboratory in 0.9% NaCl solution (Merck, Darmstadt Germany) supplemented with 100 IU/mL penicillin G (Pharmaceutical Industries, Meiji Indonesia) and 100 µg/mL streptomycin sulphate (Pharmaceutical Industries, Meiji Indonesia). The follicular fluid was aspirated using an 18 G needle connected to a 5 mL disposable syringe. The follicles as sources of IGF-I were grouped into 4 categories: follicle with diameter (Ø) of smaller than 4 mm, 4≤Ø<6 mm, 6≤Ø<8 mm, and Ø≥8 mm based on reproductive cycle (luteal and follicular phases). The pool of FF obtained in each category was centrifuged at 1500 g for 30 min at 24 ^{o}C (Park *et al*., 2009). The supernatant was filtered through a 0.20 µm filter (Sartorius Stedim, Minisart Biotech), and then aliquoted into 2 mL microtubes and stored at -20 ^{o}C until being used and analyzed. The concentration of IGF-I in FF was analyzed using KIT IGF-I 600 ELISA, EIA-4140 (DRG Instruments GmbH, Germany).

Oocyte Collection and *in Vitro* Maturation

Cumulus oocyte complexes (COCs) were recovered by slicing technique using phosphate buffered saline (Gibco by life technologies, USA) supplemented with 0.2% bovine serum albumin (BSA) (Sigma-Aldrich, USA). Oocytes selection was performed with a stereomicroscope (Olympus SZ51, Japan). Only oocytes with a homogeneous cytoplasm and surrounded by more than three layers of cumulus cells were used. The selected COCs were cultured in maturation media M199 (Gibco, USA) supplemented with 10 IU/mL of pregnant mare serum gonadotrophin (PMSG, Folligon, Intervet International B.V. Boxmeer, The Netherlands), 10 IU/mL of human chorionic gonadotrophin (hCG, Chorulon, Intervet International B.V. The European Union), 50 µg/mL of gentamycin (Sigma-Aldrich, USA), without BSA supplementation (-BSA), supplemented with 0.3% BSA (+BSA), and 10% FF (v/v) from the follicle with diameter Ø<4 mm, 4≤Ø<6 mm, 6≤Ø<8 mm, and Ø≥8 mm during the luteal phase. Oocytes maturation was done in 100 µL droplets (approximately 10–15 COCs) in petridishes with diameter of 35 mm (Nunclon, Denmark) under mineral oil (Sigma-Aldrich, USA) and incubated for 24 h at 38.5 °C in 5% CO_2 in air (Modification of Pereira *et al*., 2013).

Evaluation of Nuclear Maturation Rate

A total of 553 oocytes were processed to determine the nuclear maturation rate with the supplementation of FF in maturation media. After 22-24 h maturation period, cumulus cells were denuded from the oocyte by repeated pipetting in PBS containing 0.25% hyaluronidase (Sigma-Aldrich, USA). Denuded oocytes were then mounted on a slide and overlaid with a cover slip supported by vaselin stripes. The preparation was put into a fixative solution containing acetic acid and ethanol (1:3 v/v) for 3 d. The preparation was stained with 2% acetoorcein and rinsed with 25% acetic acid. The stained oocytes were examined under the epi-fluorescence microscope (Zeiss Axio Imager A2 with a Zeiss Axiocam HRc, Germany). Evaluation of the nuclear maturation was classified by chronological meiosis changes such as germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), anaphase I (AI), telophase I (TI), or metaphase II (MII) (Shirazi & Sadeghi, 2007). The nuclear maturation rate was calculated based on the percentage of oocytes reached the stage of metaphase II (MII) (Bijttebier *et al*., 2008).

In Vitro Fertilization

A total of 552 oocytes were used to determine the effects of FF supplementation in maturation media on fertilization rate. Frozen semen collected from one Bali cattle bull was used for *in vitro* fertilization. Frozen semen was thawed at 37 °C for 20 s and then centrifuged at 700 g for 5 min in fertilization media (Suzuki *et al*., 2000). After centrifugation, the sperm pellet was diluted in fertilization media to obtain final concentration of 1.5×10^6 mL^{-1}. Oocytes fertilization was done in 100 µL drop fertilization media under mineral oil (Sigma-Aldrich, USA) and incubated for 14-16 h at 38.5 °C in 5% CO_2. After incubation period, evaluation of the fertilization rate was done in accordance with the staining procedure for the nuclear maturation. The fertilization rate was calculated based on the number and pronuclear (PN) formation.

Statistical Analysis

The experiments of IGF-I concentration and fertilization rate were replicated 8 times, meanwhile the experiment of nuclear maturation rate were replicated seven times. All data are presented as mean ± standard error of the mean (SEM) and analyzed by ANOVA. When there was a significant effect by ANOVA, the differences among means were analyzed by Duncan's test. A significance level of 5% was considered to indicate a statistically meaningful difference. All statistical analyses were performed using the statistical package SPSS for windows version 21.

RESULTS

IGF-I Concentration in FF Bali Cattle

The concentrations of IGF-I in the follicular fluid of Bali cattle with diameter Ø<4 mm, 4≤Ø<6 mm, 6≤Ø<8 mm, and Ø ≥8 mm during the luteal phase were significantly higher (P<0.05) than those during the follicular phase (Table 1). Results of the experiment indicated that IGF-I concentrations in the follicles with diameters smaller than 6 mm had significantly higher (P<0.05) compared to those in the follicle diameters larger than 6 mm. Meanwhile, the concentrations of IGF-I in the follicular fluid obtained from the ovaries in the follicular phase did not show a significant effect of follicle diameter.

Nuclear Maturation Rate

Evaluation of the nuclear maturation was classified by chronological meiosis changes such as the stage of germinal vesicle (GV) to metaphase II (MII) after 22-24 h of maturation period (Figure 1). The percentages of oocytes reached MII in the media supplemented with

Table 1. Concentrations of IGF-I in the follicular fluid of Bali cattle with different follicle diameters and reproductive cycles*

Follicle diameter (Ø mm)	Concentration of IGF-I (ng/mL)	
	Luteal phase	Follicular phase
Ø<4	125.6± 9.6^{aA}	74.6±14.2^{B}
4≤Ø<6	137.9±10.8^{aA}	72.2±10.7^{B}
6≤Ø<8	96.8± 7.3^{bA}	62.7±13.4^{B}
Ø≥8	85.5± 3.9^{bA}	47.2±14.9^{B}

Note: *Concentrations are expressed as mean ± SEM. Means in the same rows (A,B) and columns (a,b) with different superscript differ significantly (P<0.05).

BSA and FF were significantly higher (P<0.05) compared to those cultured in the media without BSA. However, when the oocyte were cultured or matured in the media supplemented with BSA and FF, the percentage of maturation was similar among the groups, except in those cultured in the media added with FF from follicles with diameters <4 mm. The percentage of oocytes reaching MII in oocytes cultured with FF from follicle with diameter <4 mm was significantly higher (P<0.05) compared to the other groups (Table 2).

Fertilization Rate

Fertilization rate was calculated based on the number and pronuclear (PN) formation after 14-16 h of fertilization period (Figure 2). The supplementations of maturation media with BSA and FF were able to improve fertilization rate significantly (P<0.05) compared to media without BSA. The formation of 2 PN were higher (P<0.05) in oocytes cultured in the media supplemented with BSA and FF compared to those in the media without BSA. However, the supplementation of the media culture with FF derived from follicles with diameters <8 mm resulted in higher 2 PN formation compared to those derived from follicles with diameters ≥8 mm (Table 3).

DISCUSSION

IGF-I Concentration in FF Bali Cattle

Follicular fluid contains a variety of nutrients including growth factors secreted by the follicles cells (Nandi *et al*., 2008). Insulin-like growth factor-I, one of

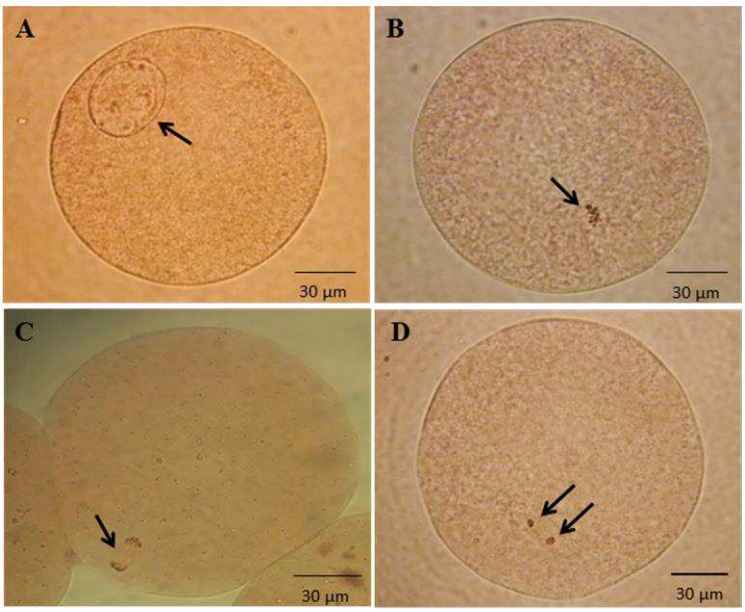

Figure 1. Bovine oocytes after 22-24 hours *in vitro* maturation period. A= germinal vesicle, B= metaphase I, C= anaphase/telophase, D= metaphase II, (arrow). Pictures of oocytes stained by 2% aceto-orcein and examined under a microscope epi-fluorescence (Zeiss Axio Imager A2 with a Zeiss Axiocam HRc, Germany).

Table 2. Nuclear maturation rate of bali cattle oocytes in maturation media supplemented with follicular fluid obtained from different diameters of follicle*

Treatments	No. of Oocytes	Nuclear maturation rate (%)					
		GV	GVBD	MI	AI/TI	MII	Degenerated
- BSA	107	0(0.0±0.0)	2(1.9±1.4)	29(27.1±1.6)^{a}	1(0.9±0.9)	68(63.6±1.9)^{a}	7(6.5±2.1)
+ BSA	95	2(2.1±1.6)	0(0.0±0.0)	19(20.0±3.6)^{ab}	0(0.0±0.0)	71(74.7±2.8)^{b}	3(3.2±2.7)
FF Ø<4	85	0(0.0±0.0)	0(0.0±0.0)	4(4.7±2.0)^{c}	0(0.0±0.0)	78(91.8±2.0)^{c}	3(3.5±2.1)
FF 4≤Ø<6	92	0(0.0±0.0)	0(0.0±0.0)	9(9.8±2.9)^{bc}	0(0.0±0.0)	76(82.6±4.1)^{bc}	7(7.6±4.7)
FF 6≤Ø<8	93	0(0.0±0.0)	0(0.0±0.0)	13(14.0±5.6)^{bc}	0(0.0±0.0)	70(75.3±3.4)^{b}	10(10.8±4.0)
FF Ø≥8	81	0(0.0±0.0)	0(0.0±0.0)	9(11.1±3.9)^{c}	0(0.0±0.0)	63(77.8±5.7)^{b}	9(11.1±5.0)

Note: *Percentages are expressed as mean ± SEM. Means in the same columns with different superscripts differ significantly (P<0.05). GV= germinal vesicle; GVBD= germinalvesicle breakdown; MI= metaphase I; AI/TI= anaphase I/telophase I; MII= metaphase II; - BSA= without supplementation of BSA; + BSA= with supplementation of BSA; FF Ø<4= follicular fluid obtained from follicle with diameter <4 mm; FF4≤Ø<6= follicular fluid obtained from follicle with diameter range of 4≤Ø<6 mm; FF6≤Ø<8= follicular fluid obtained from follicle with diameter range of 6≤Ø<8; FFØ ≥8= follicular fluid obtained from follicle with diameter range of Ø≥8 mm.

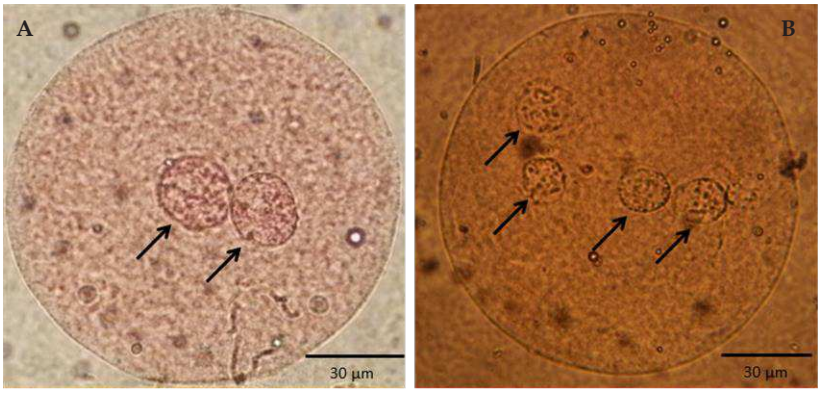

Figure 2. Bovine oocytes after 14-16 hours fertilization period. A= 2 PN and B= >2 PN. Pronuclei= PN (arrow). Pictures of oocytes stained by 2% aceto-orcein and examined under a microscope epi-fluorescence (Zeiss Axio Imager A2 with a Zeiss Axiocam HRc, Germany).

Table 3. Fertilization rate of bali cattle oocytes in maturation media supplemented with follicular fluid obtained from different diameters of follicle*

Treatments	No. of oocytes	Pronuclear formation		Fertilization rate (%)
		2 PN (%)	>2 PN (%)	
- BSA	90	29(32.2±3.9)^{a}	13(14.4±3.7)	42(46.7 ±3.8)^{a}
+ BSA	95	49(51.6 ±2.9)^{bc}	9(9.5 ±3.8)	58(61.1 ±4.8)^{b}
FF Ø<4	90	58(64.4±6.4)^{b}	5(5.6±3.3)	63(70.0±5.1)^{b}
FF 4≤ Ø<6	85	48(56.5 ±3.6)^{b}	10(11.8 ±3.4)	58(68.2±4.7)^{b}
FF 6≤ Ø<8	99	50(50.5± 2.8)^{b}	12(12.1±4.1)	62(62.6±5.1)^{b}
FFØ≥8	93	44(47.3±5.5)^{c}	10(10.8 ±4.7)	54(58.1 ±7.8)^{b}

Note: *Percentages are expressed as mean ± SEM. Means in the same columns with different superscripts differ significantly (P<0.05). PN= pronuclei; - BSA= without supplementation of BSA; + BSA= with supplementation of BSA; FF Ø<4= follicular fluid obtained from follicle with diameter <4 mm; FF4≤Ø<6= follicular fluid obtained from follicle with diameter range of 4≤Ø<6 mm; FF6≤Ø<8= follicular fluid obtained from follicle with diameter range of 6≤Ø<8; FFØ ≥8= follicular fluid obtained from follicle with diameter range of Ø≥8 mm.

complex components of IGF superfamily, plays an important role in mammalian reproduction (Velazquez *et al*., 2008). Coleman *et al*. (2007) reported that Insulin-like growth factor-I in the follicular fluid was produced during follicular growth as indicated by the IGF-I receptor expressions in the cumulus cells, granulosa cells, and theca cells. Whereas others studies reported that IGF-I in the follicular fluid was mainly produced by the follicle and corpus luteum (Woad *et al*., 2000; Velazquez *et al*., 2008), and its concentration increased progressively during the follicular growth until the ovulation of dominant follicle.

In our study, we found that the concentrations of IGF-I in follicular fluid collected from follicles with diameters of less than 4 mm to more than 8 mm during follicular phase were similar (P>0.05). On the other hand, at the luteal phase, the IGF-I concentrations in the follicular fluid collected from follicle with diameter ≥6 mm was significantly lower (P<0.05) than those collected from follicle with diameter <6 mm. These results were probably due to the increased size of the follicle during follicular growth in accordance with the decreased size and function of the corpus luteum, therefore the secretion of IGF-I in corpus luteum also decreased. This result is different from those reported previously by Oberlender *et al*. (2013) that the concentrations of IGF-I in large follicles were greater than in small follicles.

By comparing different phases of the ovarian reproductive status, we found that IGF-I concentrations in all follicles diameters during the luteal phase was higher than during the follicular phase (P<0.05). These results might be due to the contributions of the corpus luteum and the follicles to secrete IGF-I during the luteal phase of reproductive cycle. This assumption is consistent with previous report by Woad *et al*. (2000) that the bovine corpus luteum showed the expression of IGF-I receptor mRNA and a site of IGF-I reception and production. Insulin-like growth factor-I produced in the corpus luteum may enter the FF through the blood vessels of the follicle having increased vascularization and permeability during follicular development. Hurk & Zhao (2005) described that high IGF-I concentration made the follicle more sensitive to LH that result in the formation of angiogenic factors like vascular endothelial growth factor (VEGF). Vascular endothelial growth factor increases vascularization and permeability of the blood vessels. On the other hand, IGF-I plays roles to stimulate progesterone production by the corpus luteum so that during the luteal phase the higher concentrations of IGF-I is required.

Nuclear Maturation Rate

Data on the maturation rate in this study showed that only FF derived from follicle with diameter <4 mm that was able to improve the percentage of oocytes reaching the MII stage. These different results may be due to the differences in the concentrations of IGF-I in FF obtained from follicles with different sizes. In this study, we used follicular fluid from luteal phase, in which the concentration of IGF-I in follicular fluid obtained from follicle with diameter <4 mm (125.6±9.6 ng/mL) was higher than the other diameters. Oberlender *et al*. (2013) reported that the optimum concentrations of IGF-I to have positive effects on maturation rate were 129 ng/mL. Hurk & Zhao (2005) reported that the role of IGF-I is to resume meiosis by stimulating the formation of LH receptors on the granulosa cells that causes the follicle more responsive to LH. It is further explained that the process of oocyte maturation is a response to the LH surge that causes some changes in the regulatory pathway in the oocyte (Gordo *et al*., 2001). The mechanism for meiotic resumption begins with the activation of G protein that further activates phospholipase C that hydrolases phosphatidylinositol 4,5-bisphosphate (PIP2) to form inositol triphosphate (IP3) causing the mobilization of intracellular Ca^{2+} followed by an influx of extracellular Ca^{2+} (Ajduk *et al.*, 2008). The influx of extracellular Ca^{2+} in addition to inhibit adenylyl cyclase which causes lower of cAMP/PKA, also enable calmodulin-dependent protein kinase (CaM kinase II) to modify/activate the maturation promoting factor (MPF) (Hurk & Zhao, 2005; Oh *et al*., 2010; Conti *et al*., 2012).

Fertilization Rate

The success of fertilization could be assessed by the formation of male and female pro-nucleus. In this study, we found that the fertilization rate of oocytes only about 70.0% even though the maturation rate of oocyte matured in the media supplemented with follicular fluid was high (75.3%-91.8%). These results revealed that the supplementation of the maturation media with follicular fluid did not influence fertilization rate of oocytes. It was suspected that the IGF-I in the follicular fluid only acted on promoting oocyte maturation (Oberlender *et al*., 2013). The ability of oocyte to be fertilized is not only determined by the nuclear maturation of oocytes but also by the cytoplasmic maturation that are required for the progress of the maturation and to prevent polyspermy (Hyttel *et al*., 1997). The cytoplasmic maturation is influenced by several factors including the successive transformations of mitochondria, cortical granules, and smooth and rough endoplasmic reticulum (Hyttel *et al*., 1997). Furthermore, the distributions of organelles in the cytoplasm are closely correlated with the maturation and competence of oocyte (Brevini *et al*., 2007). Therefore, incomplete cytoplasmic maturation of oocytes at the MII stage may be one of reasons for the low rate of *in vitro* embryo production (Blanco *et al*., 2011). This is in contrary to previous reports that the follicular fluid promotes cytoplasmic maturation of oocyte during IVM (Hong & Lee, 2007; Papanikolaou *et al*., 2008; Grupen & Armstrong, 2010). Agung *et al*. (2013) reported that the use of porcine follicular fluid as a sole maturation media resulted in the formation of male pronuclear after in vitro fertilization in the matured porcine oocytes. Furthermore, Cruz *et al*. (2014) reported that the addition of follicular fluid at lower concentration in maturation media enhanced the total cell numbers in bovine embryos produced *in vitro*.

CONCLUSION

The concentration of IGF-I in follicular fluid obtained from the ovary in the luteal phase was higher than that in the follicular phase. The IGF-I concentration in a smaller follicle (diameter <6 mm) was higher compared to a large follicle (diameter ≥6 mm). The supplementation of follicular fluid improves the nuclear maturation and fertilization rate of oocytes of Bali cattle.

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